SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ECOLOGY
AGRI-ECOLOGY

Received
16 September 2013

Accepted
14 January 2014

Published
7 February 2014

Correspondence and requests for materials should be addressed to D.W.R. (roubikd@si.edu)

Transgenic soybean pollen (*Glycine max* L.) in honey from the Yucatán peninsula, Mexico

R. Villanueva-Gutiérrez[1], C. Echazarreta-González[2], D. W. Roubik[3] & Y. B. Moguel-Ordóñez[4]

[1]El Colegio de la Frontera Sur, Ave. Centenario km 5.5, C. P. 77014, Chetumal, Quintana Roo, México, [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, México, [3]Smithsonian Tropical Research Institute, Republic of Panama, [4]CE Mocochá, CIR Sureste, Instituto Nacional de Investigaciones Forestales, Agropecuarias y Pecuarias (INIFAP), Campeche, México.

Using precise pollen species determination by conventional microscopic methods, accompanied by molecular genetic markers, we found bees collect GMO (genetically modified) soybean pollen and incorporate it in Yucatan honey. Honey comb samples from Las Flores, Campeche, Mexico, often contained soybean pollen. Pollen in honey was analyzed in nine samples; six contained substantial soy pollen and two tested positive for soybean GMO. Our analyses confirm field observations that honey bees, *Apis mellifera*, gather soybean pollen and nectar. The resultant risk for honey production in the Yucatán Peninsula and Mexico is evident in wholesale price reduction of 12% when GMO products are detected and honey consignments are rejected. Although this affects only 1% of current export honey (2011–2013) GMO soybean is an unacknowledged threat to apiculture and its economics in one of the world's foremost honey producing areas.

When in flower, GMO (genetically modified) crops provide resources gathered by bees. As also mistakenly inferred for coffee[1], the fact that soybean plants can self-pollinate is claimed to mean they are not visited or pollinated by bees in Mexico[2–5]. There is, however, ample evidence regarding *Apis mellifera* as a flower visitor of this major crop[6–9]. We found direct evidence from identification of pollen in honey that honey bees both visit and likely pollinate Yucatán peninsula soybean crops. We worked in Hopelchén, Campeche (where approximately 10 thousand ha of soybean were cultivated in 2012), to determine if GMO pollen from cultivated soya is contained in marketable honey, where apiculture is a major economic activity. Senasica (2012) authorized Monsanto for the commercial cultivation of 253,500 ha GM soybean in 7 states of Mexico, 60,000 ha within the Yucatán peninsula (Campeche, Yucatán and Quintana Roo states)[5]. Although several NGOs suspended legal permission for GM soybean cultivation, at least in Hopelchén, Campeche, 10,000 ha were cultivated in 2012, mixed with non-transgenic soybean and sorghum.

As background, it is significant that on September 6, 2011, the Court of Justice of the European Union established three conditions concerning transgenic, or GMO, pollen in a honey sample. (Commercial honey analysis now includes markers that detect both accepted and unacceptable for human consumption GMO plant products[10], and in Europe, appropriate labeling has been required since 2003). If from a GM crop unapproved for human consumption, it cannot be marketed in the EU. [This includes certain soy and other animal feed]. If from an approved GM for human consumption, if 0.9% or more of pollen in honey is GMO, honey can be imported, but the label should specify that it contains GM ingredients. [This often means it cannot be marketed as an 'organic' product]. If an approved crop with transgenic pollen is represented by less than 0.9% of the total in honey [currently subject to interpretation that varies from pollen grain number to species proportion, both in multi-species honey], it can be marketed without any restriction. [Like the preceding category, the honey or product is not deemed 'organic' and no further quantification or analysis may be performed.]

Such provisions require analysis of honey samples from Mexico and consequently affect costs. As we detail below, the sale of honey consignments to Germany is more strongly affected than the above rules would suggest. Any kind of GMO product in honey, in amount as small as a single pollen grain within a sample from a consignment ready for export, causes that shipment to be rejected. More than 10,000 ha of the leguminous crop soybean (*Glycine max* L.) are in our study area, with some sorghum (Fig. 1). Beekeeping with the Africanized honey bee is extensive in the Yucatan peninsula, which produces 20,807 tons of honey per year, and considerable

Figure 1 | Soybean field crop with forest patch in the center. The lowland forest of this region is of low stature, with a canopy less than 20 m high, and is continuous (note background), unless cleared for agriculture or livestock. Photograph by R. Villanueva-Gutiérrez.

wax, from 620,521 colonies[11]. What is not commonly understood is that such colonies are environmental monitors, each having a range of some 200 km^2 in which they gather floral resources[12]. Combined with the powerful method of pollen identification of the highly diverse plants of tropical forest regions, developed first in Panama and then in the Yucatán, Mexico[13,14], the honey bees may be used as indicators of many features in the biotic environment –including GMO plants.

Results

Of nine samples analyzed, pollen grains of soybean occurred in six–taken where the bees foraged within range of soybean plots. Honey bee foraging range is well known to routinely encompass several km, but the consideration of distance from apiary to cultivated plot also provided a check on the results. All but one of the colonies sampled within foraging range of soybean contained its pollen, and no soybean pollen was found from colonies far from their fields (Table 1). Soybean was 8 to 48% of pollen from all sources in honey samples (Table 1), far above permitted levels for a 'natural' native honey. The soybean honey was sent to Intertek laboratory, Bremen, Germany to test for Genetically Modified Organisms. Results of PCR (polymerase chain reaction) analysis confirmed two samples positive for soybean transgenic material (Table 1), and no further identifications as to cultivar or whether permitted or not for human consumption were made. However, all GMO soybean grown in Campeche state is classified as fit for human consumption (Federico Berrón, APIARIOS TAL, personal communication, December 6, 2013). The results of our analyses demonstrate that bees visit the flowers of soybean for nectar and/or pollen in the Yucatán peninsula, thus the pollen of GMO soybean flowers is also contained in honey harvested by beekeepers.

Several other honey and pollen sources appeared in honey, demonstrating that bees foraged widely and had access to different floral species. In honey sample No. 2 we found that the most abundant pollen grains were *Chamaesyce*, *Viguiera dentata*, *Croton*, *Eleocharis cellulose, Chenopodium ambrosioides*, and *Thouinia canescens* (Fig. 2). In sample No. 7 the most abundant were *Glycine max*, *Viguiera dentata, Eleocharis cellulose, Evolvulus sericeus, Parthenium histerophorus* and *Conyza bonariensis* which provided bees with nectar or pollen.

In soybean, pollen grains are spherical, psilate and tricolporate, of 20 μ in diameter. Some pollen grains found in the honey samples, like *Desmodium, Lonchocarpus* and *Nissolia*, have a morphology similar to *Glycine max* pollen grains, but are larger and the pores have a different structure[14].

Discussion

Honey samples contained native nectar and pollen resources for Africanized honey bees in the disturbed areas of low forest in Quintana Roo State[15,16]. Bees nonetheless harvested cultivated GM soybean flowers in two of the observed bee yards within flight range of such crops. Previous study of another legume, in Kenya, demonstrates that its bee visitor, *Xylocopa*, flies distances of at least 7 km, and carries GMO pollen from cultivated to wild cowpea flowers[17]. That study utilized aerial surveillance and transmitters affixed to the bees, to map their large-scale movement between crops and wildlands. Our pollen taxonomy provides comparable evidence of flights from honey bee hives to cultivated plants. The honey bee and many other bees[18] are capable of flight reaching several to many km from their home base, but most are quite small and forage over a wide range, among trees and other landscape features, thus cannot be monitored with transmitters. However, they may nonetheless transmit pollen between nestmates, making a 'horizontal bee transfer' which may greatly extend pollen dispersal[19]. There was no conceivable contamination with non-foraged soybean in the present study. One of the questions made to beekeepers at the beginning of the work was whether they fed their colonies food supplements. Their reply was negative. Because soy-based protein supplements are sometimes provided for bee colonies by beekeepers, this question has particular relevance. Beekeepers do not have to feed their colonies with supplemental food because bees find sufficient nectar and pollen all year in the Yucatán peninsula.

Gallez et al.[6] made a pollen analysis of 36 honey samples from Argentina and determined that *Glycine max* was present in 100% of them. Other researchers[7–9] conclude that the Africanized honey bee pollinates soybean and increases seed production. The Africanized honey bee often prefers rosid floral resources such as the legumes, and forages widely on them[20] thus may be expected to utilize *G. max*. It is noteworthy that certain agencies, such as those we cite for

Table 1 | Quantification of soybean pollen and detection of Genetically Modified Organisms in honey samples from Mexican apiaries in soybean cultivation areas. Samples were taken in the municipality of Hopelchén, state of Campeche (see text)

Sample No.	No. of pollen grains per 10 g of honey	Detection of Genetically Modified soybean	Site	Beekeeper	Distance between apiary and soybean crop
1	0	−	Las Flores	1	300 m
2	28	+	Las Flores	1	300 m
3	56	−	Las Flores	2	150 m
4	105	−	Las Flores	3	80 m
5	132	−	Las Flores	4	100 m
6	68	−	Las Flores	1	40 m
7	51	+	Las Flores	5	300 m
8	0	−	Ejido Chencó	4	48 km
9	0	−	Ejido Chencó	4	40 km

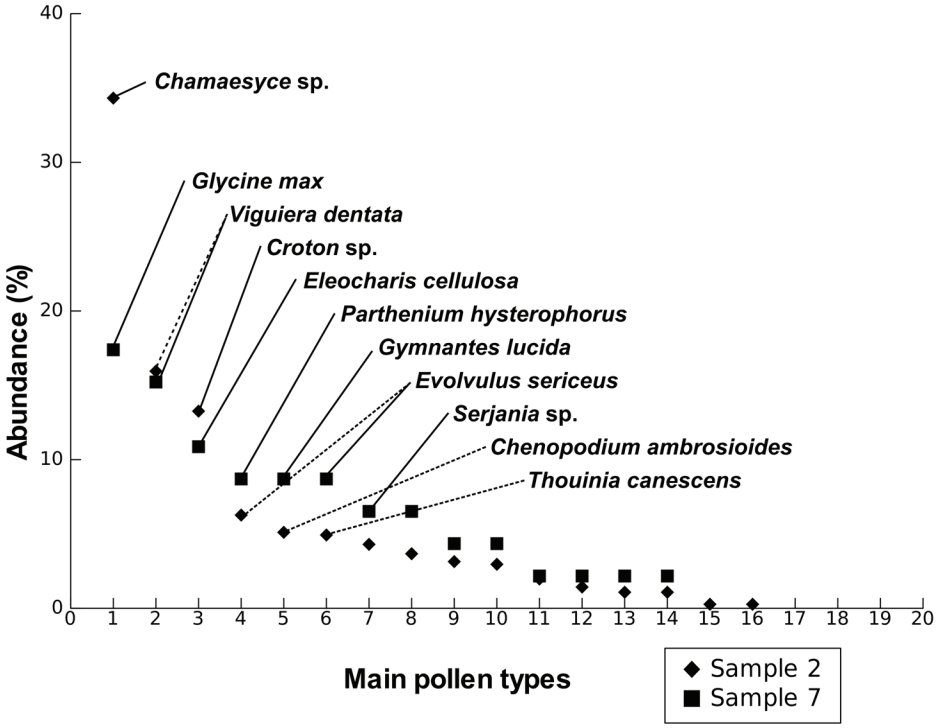

Figure 2 | Pollen types found in representative honey samples No. 2 and 7, Las Flores, Campeche. Sample taken from beekeepers Feliciano Ucán and Emiliano Huchin, respectively.The most common pollen types are indicated with their relative abundance.

Mexico[2] continue to misinterpret plant reproductive traits such as autogamy or self-pollination. As shown previously for other 'selfing' crops, this trait indicates nothing about visits to their flowers by animals seeking food[1]. Mexico is the fourth largest producer and fifth largest exporter of honey in the world, and third largest in bee wax production (data from 2011; faostat3.fao.org)[11,21]. Honey exported to Europe, mainly to Germany, is unacceptable if contaminated with transgenic pollen grains, regardless of whether or not they are from a GM product approved for human consumption (Federico Berrón, personal communication, December 6, 2013). Alternate pricing to importers follows the USA standard of $3000 per metric ton, 12% less than prices paid by European Union importers; this has affected 420 metric tons, approximately 1% total Yucatan peninsula production in the past two years (Federico Berrón, personal communication).

Given a range of PCR technologies, such as barcoding and other techniques[10,22] that readily detect such pollen in commercial honey, the economic justification for using GMO crops in productive organic and commercial honey regions must be carefully weighed against the advantages given for GMO applications[2]. Although our results are from only nine honey samples of an area from the Yucatán Peninsula, we demonstrate that honey bees frequently visit the flowers of GM soybean in Campeche, Yucatán peninsula, and suggest other Mexican honey contains transgenic pollen grains of this particular cultivar. With an economy based on subsistence agriculture associated with honey production, the social implications of this shift in quality and status of Yucatán honey are likely to be contentious and have profound implications for beekeeping in general.

Methods

Honey samples from the municipality of Hopelchén, Campeche, southeastern Mexico often contained soybean pollen. Nine honey samples were obtained from the "Las Flores" and "Ejido Chencó" regional apiaries, the first on 30 September, 2012 with another on 28 October. A complete comb was obtained from the hives in order to obtain enough honey for pollen and genetic analyses (Fig. 3).

All beekeepers were asked if they were feeding their colonies with a food supplement, and the dates of honey harvest.

The "bee yards" were of 5 individual beekeepers; those of 'Las Flores' were within 300 m of soybean fields. The honey bees easily reach the soybean flowers because the mean distance of foraging honey bee flight, measured on the Africanized honey bee (*Apis mellifera*), is around 2 km[23], while the maximum radius is generally 8 km[12]. The soybean cultivation area is surrounded by a flooded forest. Honey samples were taken from nest combs and studied at the palynological laboratory of El Colegio de la Frontera Sur (ECOSUR) in Chetumal. They were processed and acetolyzed[24] with some modifications[15] and mounted on microscope slides. Pollen grains were then identified and counted, for the identification of the grains we used a pollen atlas[13,14] and the palynological reference collection of Herbario ECOSUR (CIQRO). Nine honey samples were analyzed (Table 1).

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Figure 3 | Sampling honey from the apiaries installed near soybean. The owner and technician stand with a newly removed hive frame containing pure honey sealed with beeswax, within one of the thousands of small, local apiaries maintained using Africanized honey bees now resident in the Neotropics[12]. Photograph by R. Villanueva-Gutiérrez.

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Acknowledgments

We thank Irma Gómez González for sampling Hopelchén apiaries, Federico Berrón for commercial honey data, Rodrigo Villanueva-Ceballos for his help with graphs and Wilberto Colli-Ucán and Margarito Tuz-Novelo for assistance.

Author contributions

Y.B.M.O. did field work, R.V.G. did lab work, R.V.G. and D.W.R. analyzed data, D.W.R., R.V.G. and C.E.G. wrote the paper, and all four authors reviewed the manuscript. There are no financial or other conflicts of interest.

Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Villanueva-Gutiérrez, R., Echazarreta-González, C., Roubik, D.W. & Moguel-Ordóñez, Y.B. Transgenic soybean pollen (*Glycine max* L.) in honey from the Yucatán peninsula, Mexico. *Sci. Rep.* **4**, 4022; DOI:10.1038/srep04022 (2014).